--50. An in vitro method according to claim 34, wherein the step of placing the DNA construct in an environment wherein transcription can occur comprises transfecting an eukaryotic cell with a plasmid comprising the DNA construct.--

--51. An in vitro method according to claim 1, wherein the six copies of the enhancer element are upstream of the promoter.--

--52. The method according to claim 2, wherein the enhancer element consists of the nucleotide sequence TTCTGAGAA.--

--53. An enhancer according to claim 5, consisting of the nucleotide sequence TTCTGAGAA.--

--54. An isolated DNA construct according to claim 44, further comprising a structural gene.--

## REMARKS

The Official Action dated April 26, 2000 has been carefully considered. Accordingly, the changes presented herewith, taken with the following remarks, are believed to be sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

Claims 22, 33 and 43 have been canceled. Claims 1-2, 5, 8, 10, 19, 23, 25, 27-28, 30-32, 34, 41, 44, and 46-48 have been amended. Support for the amendment to claim 1 can be found on page 2, lines 18-20, Example 2 on page 3, lines 2-31 and Example 5 on page 4, lines 14-20, while support for the amendments to claims 2 and 28 can be found on page 2, lines 18-20. Support for the amendment to claims 19 and 34 can be found on Example 2 on page 3, lines 2-31 and Example 5 on page 4, lines 14-20. Support for the amendment to claim 32 can be found in original claim 33, while support for the amendment to claim 41 can be found in original claim 43. Support for the amendment to claim 44 can be found on page 3, lines 8 and 36, and page 4, lines 7 and 17, while support for the amendment to claim 46 can be found on page 3, lines 14-19 and 34-36, and page 4, lines 16-18. Claims 5, 8, 10, 23, 25, 27, 30-31 and 47-48 have been amended as to form.

Claims 49-54 have been added. Support for claims 49 and 50 can be found in Example 2 on page 3, lines 2-31 and Example 5 on page 4, lines 14-20. Support for claim 51 can be found

on page 3, lines 6-10 and page 4, lines 16-18, while support for claims 52 and 53 can be found on page 2, lines 18-19. Finally, support for claim 54 can be found in original claim 1. As the amendments to the claims are fully supported by the specification as originally filed and add no new matter, entry is believed to be in order.

The present invention is directed towards methods of enhancing the transcription of a gene in a DNA construct incorporated into the genome of a eukaryotic host cell; enhancer elements which when used in a DNA construct are responsive to hormonal stimuli; and expressions vectors comprising hormone-responsive enhancer elements.

More particularly, according to claim 1 the invention is directed to a method of enhancing the transcription of a gene in a DNA construct comprising a structural gene and a promoter wherein the DNA construct is incorporated into the genome of a eukaryotic host cell. The method comprises providing transfecting the eukaryotic host cell with six copies of an enhancer element, and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof. The enhancer element comprises the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is not the DNA sequence of nucleotide sequence SEQ ID NO:1, and is responsive to both lactogenic hormones and somatogenic hormones.

According to claim 5 the invention is directed to an enhancer element which when used in a DNA construct for transfection of a eucaryotic host cell is responsive to hormonal stimuli. The enhancer element consists essentially of the nucleotide sequence TTCTGAGAA, with the proviso that said nucleotide sequence is not the DNA sequence of the SPI-growth hormone responsive element (SPI-GHRE). The enhancer element is responsive to both lactogenic hormones and somatogenic hormones.

According to claim 8 the invention is directed to an expression vector comprising a structural gene encoding a desired protein or polypeptide and a promoter, wherein the vector further comprises six copies of an enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is not the nucleotide sequence SEQ ID NO:1.

According to claim 19 the invention is directed to an in vitro method of enhancing the transcription of a gene in a DNA construct comprising a structural gene and a promoter upstream of the structural gene. The method comprises placing the DNA construct in an eukaryotic host

cell wherein transcription can occur; transfecting the eukaryotic host cell with at least one enhancer element consisting of the nucleotide sequence TTCTGAGAA; and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof.

According to claim 23 the invention is directed to an enhancer element which is responsive to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof when the enhancer element is used in a DNA construct for transfection of a eukaryotic host cell. The enhancer element consists essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence of SEQ ID NO:1.

According to claim 27 the invention is directed to an expression vector comprising a structural gene encoding a protein, a promoter, and at least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence SEQ ID NO:1.

According to claim 30, the invention is additionally directed to a DNA comprising a promoter, a structural gene, and at least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence SEQ ID NO:1.

According to claim 34 the invention is directed to an in vitro method of enhancing the transcription of a gene in a DNA construct comprising a structural gene, a promoter and at least one enhancer. The method comprises placing the DNA construct in an environment wherein transcription can occur; and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof. The enhancer element consists essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence SEQ ID NO:1.

According to claim 41 the invention is directed to an in vitro method of enhancing transcription of a structural gene, comprising the steps of preparing a plasmid DNA construct comprising a structural gene, a promoter upstream of the structural gene, and at least one enhancer consisting of the sequence TTCTGAGAA upstream of the promoter; transfecting a cell with the plasmid DNA construct; and exposing the cell to prolactin.

According to claim 44 the invention is directed to an isolated DNA construct comprising a promoter and six repeats of an enhancer, wherein the enhancer consists essentially of the sequence TTCTGAGAA.

According to claim 46 the invention is directed to an in vitro method of enhancing the transcription of a gene, comprising the steps of providing a cell comprising the gene; transfecting the cell with at least one copy of the nucleotide sequence TTCTGAGAA; and exposing the DNA construct to prolactin.

Claims 25-29 and 34-36 have been objected to as containing informalities. The Examiner alleges sequences recited in the claims are not identified by SEQ ID NO. The claims have been amended in accordance with the Examiner's recommendation, whereby the objection has been overcome.

Claims 1-2, 19-22, 34-36, 39-40 and 44-48 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way to enable one skilled in the art to make and/or use the invention. The Examiner alleges the specification fails to provide an enabling disclosure because the claims require that the enhancer element be placed upstream of a promoter which is already present in the genome of the host cell, which requires gene targeting methods not described in the specification. The Examiner further alleges the specification does not teach a selection step for identifying and isolating a clone with the enhancer integrated into the desired position. The Examiner alleges in the absence of specific guidance for achieving targeted integration of the enhancer element undue experimentation would be required for one skilled in the art to practice the claimed invention.

The Examiner alleges claims 44-45 are directed toward an isolated DNA construct comprising six repeats of an enhancer consisting essentially of the sequence TTCTGAGAA. The Examiner alleges the specification fails to provide an enabling disclosure for the claimed DNA construct because the specification does not teach how to use a DNA construct does not comprise a gene.

As will be set forth below, Applicants submit that claims 1-2, 19-20, 34-36, 39-40 and 44-48 are supported by an enabling specification. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

Claim 1, and claims 19 and 46 have been amended to delete the limitations of "providing upstream of said promoter six copies of an enhancer element" and "providing upstream of the

promoter at least one enhancer element", respectively, and to include the limitations "transfecting the eukaryotic host cell with six copies of an enhancer element" and "transfecting the eukaryotic host cell with at least one enhancer element", respectively.

Claim 34 has been amended to delete the limitation "providing upstream of the promoter at least one enhancer element" and has been amended to recite a method of enhancing transcription of a gene in a DNA construct comprising a structural gene, a promoter upstream of the structural gene, and at least one enhancer element upstream of the promoter, the method comprising placing the DNA construct in an environment wherein transcription can occur, and exposing the construct to a hormone.

Applicants direct the Examiner's attention to Example 2, set forth on page 3 of the specification and Examples 4 and 5, set forth on page 4 of the specification. The examples describe the transfection of eukaryotic host cells with up to six copies of an enhancer element. The examples further describe placing the DNA construct in an environment wherein transcription can occur, such as a cell, and exposing the construct to a hormone. Thus claims 1, 19, 34 and 46 are supported by an enabling disclosure.

Claim 44 recites a method directed toward an isolated DNA construct comprising a promoter and six repeats of an enhancer, wherein the enhancer consists essentially of the sequence TTCTGAGAA. Applicants direct the Examiner's attention to Example 2, page 3 of the specification. Example 2 demonstrates that a DNA construct comprising a promoter and six repeats of an enhancer may be used to prepare an expression plasmid containing a recombinant hormone responsive reporter consisting of six repeats of an enhancer element and a promoter. Example 2 further discloses that techniques to make such vectors are well known to those in the art. Thus, the specification discloses a use for a DNA construct in accordance with claim 44. Further, claim 54 recites an isolated DNA construct in accordance with claim 44, further comprising a structural gene.

Therefore, for the reasons set forth above, Applicants submit that claim 1 and claims 2 and 39-40 dependent thereon, claim 19 and claims 20-22 dependent thereon, claim 34, and claims 35-36 dependent thereon, claim 44 and claim 45 dependent thereon, and claim 46 and claim 47 dependent thereon, are supported by an enabling specification, whereby the rejection should be reversed.

Claims 1-2, 5, 7-11, 15-17, 19-24, 27, 34-36, 39-40, and 46-48 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Examiner alleges claims 1, 2, 19-22, 34-36, 39, 40 and 46-48 have been rejected as being incomplete for omitting essential steps, that is, a transfection step for getting the enhancer element into the host cell. The Examiner alleges claims 19-22, and 34-36 are further incomplete for omitting another essential step, namely, placing the DNA construct in an environment where transcription can take place, such as a cell or cell-free extract. The Examiner alleges claims 2, 5, 7-11, 15-17, 23 and 24 are indefinite in the recitation of "SP-growth hormone responsive element" as the specification teaches the nucleotide sequence element is a 50 base pairs element and has the sequence of SEQ ID NO: 1, however, SEQ ID NO:1 contains 52 base pairs. The Examiner alleges claim 27 is indefinite in its recitation of "structural" protein as the specification does not define "structural protein".

As will be set forth below, Applicants submit that claims 1-2, 5, 7-11, 15-17, 19-21, 23-24, 27, 34-36, 39-40 and 46-48 are definite. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

Claim 1 recites the step of transfecting the eukaryotic host cell with six copies of an enhancer element, while claim 19 recites the steps of placing the DNA construct comprising a structural gene and a promoter in an eukaryotic host cell wherein transcription can occur and transfecting the eukaryotic host cell with at least one enhancer element. Claim 34 recites the step of placing the DNA construct comprising a structural gene, a promoter and at least one enhancer in an environment wherein transcription can occur, while claim 46 recites the steps of steps of providing a cell comprising the gene and a promoter and transfecting the cell with a DNA construct comprising at least one copy of the nucleotide sequence.

Thus claims 1, 19, 34 and 46 include the steps alleged by the Examiner to be essential, and claim 1 and claims 2 and 39-40 dependent thereof, claim 19 and claims 20-21 dependent thereon, claim 34 and claims 35-36 dependent thereon, and claim 46 and claim 47-48 dependent thereon are complete and definite.

Claims 2, 5 8 and 23 do not recite the recitation "SP-growth hormone responsive element", and instead recite enhancer elements consisting essentially of nucleotide sequence TTCTGAGAA, wherein the nucleotide sequence is not the DNA sequence of SEQ ID NO:1. Thus, claim 2, claim 5 and claims 7 and 15 dependent thereon, claim 8 and claims 9-11 and 16-17 dependent thereon, and claim 23 and claim 24 dependent thereon, are definite.

Claim 27 has been amended to recite an expression vector comprising a structural gene, a promoter, and at least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, wherein the nucleotide sequences other than SEQ ID NO:1. Thus claim 27 is definite.

Therefore, for the reasons set forth above, Applicants submit that claims 1-2, 5, 11-17, 19-21, 23-24, 27, 34-36, 39-40 and 46-48 are definite whereby the rejection of these claims under 35 U.S.C. §112, second paragraph, should be withdrawn.

Claims 2, 19-20 and 41-42 have been rejected under 35 U.S.C. § 102 as being anticipated by Yoon et al., *J. Biol. Chem.*, 265:19947-19954 (1990). The Examiner alleges Yoon et al. teach transcription of serine protease inhibitor (SPI 2.1) gene is induced by growth hormone, and that Yoon et al. further teach the isolation and characterization of the SPI 2.1 gene from rat genomic library. The Examiner alleges Yoon et al. teach that portions of the 5′-flanking region of the gene were fused to a heterologous promoter and reporter gene and introduced into rat hepatocytes thereby generating expression vectors and eukaryotic host cells. The Examiner further alleges that Yoon et al. teach that essential sequences in the segment from -275 to -54 in the SPI 2.1 sequence could confer growth-hormone responsiveness when linked in tandem, and that Yoon et al. teach reporter constructs containing six tandem copies of SPI-GHRE linked to a thymidine kinase promoter and a chloramphenicol acetyl transferase coding sequence.

As will be set forth below, Applicants submit that claims 2, 19-20 and 41-42 are not anticipated by Yoon et al. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

Yoon et al. teach that transcription of the SPI 2.1 gene is induced by growth hormone, and that when portions of the 5′-flanking region are fused to heterologous promoter and reporter genes introduced into hepatocytes, there is a 2 to 3 fold induction of reported gene activity in cells grown in the presence of growth hormone. Yoon et al. also teach that further definition of the essential sequences reveal that a segment from -147 to -102 could confer growth hormone responsiveness, and sets forth the nucleotide sequence for this segment.

Applicants find no teaching or suggestion in Yoon et al. of a segment smaller than the 50 bp segment set forth as SPI-GHRE which is responsive to growth hormone. Yoon et al. disclose 50 to 55, 38 and 32 bp products formed using primer extension and RNase protection methods. However, these groups of products which result from primer extension do not suggest any DNA

sequence smaller than the SPI-GHRE set forth in Yoon et al. would confer growth hormone responsiveness to a DNA construct.

More particularly, Applicants find no teaching or suggestion in Yoon et al. of an enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, wherein the nucleotide sequence is not the DNA sequence of nucleotide sequence SEQ ID NO:1, as recited in claim 2, or of an enhancer element consisting of the nucleotide sequence TTCTGAGAA, as recited in claims 9 and 41, upon which claims 20 and 42 depend, respectfully.

Further, Applicants find no teaching or suggestion in Yoon et al. of an in vitro method comprising the combination of steps of preparing a plasmid DNA construct comprising a structural gene, a promoter, and at least one enhancer consisting of the nucleotide sequence TTCTGAGAA, transfecting a cell with the plasmid DNA construct, and exposing the cell to prolactin.

Therefore, for the reasons set forth above, Applicants submit that claims 2, 19-20 and 41-42 are not anticipated by Yoon et al., whereby the rejection should be withdrawn.

Claims 5, 7, 15 and 23-33 have been rejected as obvious over Lindquester et al., *Nucleic Acids Research*, 17 (5): 2099-2118 (1989). The Examiner alleges that Lindquester et al. disclose the nucleic acid sequence of avian tropomyosin gene, and the gene includes the sequence TTCTGAGAA in one of the introns. The Examiner further alleges that since enhancer elements are known to be located in introns, the presence of the sequence TTCTGAGAA in the intron would permit it to function as an enhancer element, and that hormone responsiveness of the element is an inherent property of the element. The Examiner further alleges that one would have been motivated to construct an expression vector comprising the tropomyosin gene and a host cell comprising the expression vector in order to produce the tropomyosin protein in culture.

As will be set forth below, Applicants submit that claims 5, 7, 15 and 23-32 are not rendered obvious by Lindquester et al. Accordingly, the rejection is traversed and reconsideration is respectfully requested.

Lindquester et al. disclose a tropomyosin gene consisting of 13 exons spaced over about 18 kilobase pairs of DNA. More specifically, Figure 1B of Lindquester et al. sets forth the nucleotide sequence of alpha-tropomyosin gene wherein nucleotide position plus one marks the transcription start site, and the sequence ranges from -427 to 20379.

Although Lindquester et al. disclose the avian tropomyosin gene comprises introns, Applicants find no teaching or suggestion in Lindquester et al. which, if any, introns serve as enhancers as opposed to serving as promoters or having no apparent functionality. Thus, although an intron of the avian tropomyosin gene disclosed by Lindquester et al. comprises the sequence TTCTGAGAA, one of ordinary skill would have no reason to assume such a intron contains any enhancing activity and, consequently, one would have no motivation to use a sequence consisting of, or consisting essentially of, TTCTGAGAA as an enhancer, and would have no reasonable expectation of successfully enhancing transcription using such as sequence.

The Examiner alleges that Lindquester et al. need not teach that the DNA sequence TTCTGAGAA functions as an enhancer or as responsive to hormones, that the reference provides motivation for making an expression vector to express tropomyosin in culture, and that the hormone responsiveness conferred by the presence of the element is an inherent property of the element. However, it is well settled that the question of obviousness under 35 U.S.C. §103 is not what the routineer could have done, but rather what would have been obvious for such a person to do, *Orthokinetics Inc. v. Safety Travel Chairs, Inc.*, 1 USPQ2d 1081 (Fed. Cir. 1986). Further to establish a *prima facie* case of obviousness, three basic criteria must be met: there must be some suggestion or motivation, either in the references themselves or the knowledge available to one of ordinary skill in the art, to modify the reference, there must be a reasonable expectation of success, and the reference must teach or suggest all of the claim limitations. MPEP 706.02(j). Inherency and obviousness are entirely different concepts and the view that success would have been inherent cannot substitute for a showing of reasonable expectation of success. *In re Rinehart*, 189 USPQ 143 (CCPA 1976).

At most the teachings of Lindquester et al. might suggest to one of ordinary skill that it may be possible to produce tropomyosin by using the entire tropomyosin gene sequence disclosed in Lindquester et al. However, Applicants find no teaching or suggestion in Lindquester et al. of utilizing a portion of a tropomyosin intron as an enhancer element in a DNA construct. Thus, even were one of ordinary skill motivated to use the entire sequence set forth in Lindquester et al. in order to produce avian tropomyosin, one of ordinary skill would have no motivation to use a portion of a single intron of the Lindquester et al. sequence with any reasonable expectation of successfully enhancing transcription. Further, one of ordinary skill would have no reasonable expectation that such a portion of an intron sequence would be responsive to both lactogenic

hormones and somatogenic hormones. Thus, Lindquester et al. do not render obvious an enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, with the proviso that said nucleotide sequence is not the DNA sequence of SEQ ID NO:1, as required by claims 5, 7, 15, 23-25, 27 and 30-31, or an enhancer element consisting of the nucleotide sequence TTCTGAGAA, as required by claims 26, 28 and 32.

Therefore, for the reasons set forth above, Applicants submit that claim 5, 7, 15 and 23-32 are not rendered obvious by Lindquester et al., whereby the rejection of these claims under 35 U.S.C. §103 based on Lindquester et al. should be withdrawn.

For the reasons set forth above, Applicants submit that the claims herein are definite, supported by an enabling specification, and are neither anticipated by Yoon et al., nor rendered obvious by Lindquester et al. Therefore, the Examiner is respectfully requested to withdraw the rejections to these claims and to allow the application to pass to issue.

Respectfully submitted,
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